

Her2/neu Protein Expression and Oncogene Amplification in Gastric Carcinoma in Egyptian patients

Thesis submitted for partial fulfillment of M.D. degree in pathology

by:

Ranya Abdel Rahman Ebrahim Al Ashiry

M. Sc of pathology, Faculty of Medicine, Cairo University

Under Supervision of:

Prof. Dr. Ali Ahmed El Hindawi

Professor of Pathology, Faculty of Medicine, Cairo University

Prof. Dr. Ahmed Mahmoud Abdel Hadi

Professor of Pathology, Theodor Bilharz Research Institute

Dr. Amal Ahmed Mohamed Hareedy

Lecturer of Pathology, Faculty of Medicine, Cairo University

Faculty of Medicine

Cairo University

2015

ABSTRACT

BACKGROUND

Gastric cancer (GC) is a major cause of cancer death worldwide, especially in the developing countries. In Egypt, GC is the 12th most common cancer in both sexes representing 1.6% of the total cancers. It is the 12th leading cause of cancer death representing 2.2% of the total cancer mortality. Amplification of the Her2/neu gene and overexpression of the Her2/neu protein in GC is a golden criterion for target therapy with trastuzumab (Herceptin).

MATERIAL AND METHODS

Eighty five formalin-fixed and paraffin-embedded tumor tissue samples from Egyptian gastric carcinoma patients were studied for Her2/neu with immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) methods. Thirty cases of non-malignant lesions (Gastritis, intestinal metaplasia, adenoma with low grade dysplasia, adenoma with high grade dysplasia) were studied with IHC. Associations between clinicopathological factors and Her2/neu positivity were done.

RESULTS

Twenty three cases (27%) were defined as positive for Her2/neu gene amplification and/or protein overexpression. The levels of Her2/neu positive (3+), Her2/neu equivocal (2+) and Her2/neu negative (1+/0) were measurable in 14.2%, 32.9% and 52.9% of the samples, respectively. FISH showed that Her2/neu gene was amplified in 22 cases,

10 Her2/neu positive (3+), 11 (39.3%) Her2/neu equivocal (2+) and 1 Her2/neu negative (1+) cases with IHC staining. There was a higher Her2/neu positivity (3+) in intestinal type and mixed carcinoma, and moderately differentiated tumors.

None of the gastritis, intestinal metaplasia or adenoma with low grade dysplasia cases showed positivity for Her2/neu (3+). The Her2/neu positivity (3+) was associated with both adenocarcinoma cases and high grade dysplasia ($P = 0.002$).

CONCLUSION:

Her2/neu expression in Egyptian patients was comparable to that in other populations; 27% of Egyptian patients with primary GC and GEJ adenocarcinoma were Her2/neu-positive on IHC and FISH. Her2/neu positivity (3+) was common in the mixed, intestinal type and moderately differentiated carcinoma. The results highlight the necessity of FISH test for further categorization when gastric cancer cases are equivocal (2+) by IHC to determine eligibility for the targeted therapy. Stepwise increase in the expression of Her2/neu was seen in low grade dysplasia, high grade dysplasia and carcinoma cases implying its role in cancer evolution.

Key words:

Egyptian; Gastric cancer; Gastroesophageal cancer; Her2/neu; Protein expression (IHC); Gene amplification (FISH).

ACKNOWLEDGMENT

First and foremost "Thanks to ALLAH", the most gracious and merciful for all his innumerable blessings

*I am very much indebted to Prof. Dr. **Ali El Hindawi** Professor of Pathology, faculty of medicine, Cairo University, I would like to express my deepest gratitude, great thanks, and appreciation to him for his precious remarks and great experience that were very valuable in completing this work,*

*I would like to acknowledge my eternal indebtedness to Prof. Dr. **Ahmed Abdel Hadi**, Professor of Pathology, Theodor Bilharz Research Institute, for his invaluable guidance, continuous support, keen interest, and unlimited encouragement.*

*I would like to sincerely thank Prof. Dr. **Olfat Hammam**, Professor of Pathology, Theodor Bilharz Research Institute, that without her help and instructions it would have been very difficult to produce this study; from her I received invaluable insights, precious remarks, and valuable advices.*

*I would like to sincerely thank Dr. **Amal Hareedy** Lecturer of Pathology, Faculty of Medicine, Cairo University for the precious time and effort she offered to help me to accomplish this work,*

*I am deeply thankful to Dr. **Heba Khalil** Lecturer of Pathology, Theodor Bilharz Research Institute, who saved no time and effort in helping me in the study and for her continuous support.*

Finally I would like to thank my precious and kind family members, especially my Mom, Dad and my husband, without their love, motivation, support and encouragement, this work would have never seen light.

List of abbreviations

5-FU	5-fluorouracil drug
AJCC	American Joint Committee on Cancer
ASCO-CAP	The American Society of Clinical Oncology/College of American Pathologists.
ATP	Adenosine Triphosphate
Bax	BCL2-associated X protein
BC	Before Christ
Bcl-2	B-cell CLL/lymphoma 2
Bcl-6	B-cell CLL/lymphoma 6
BRCA1/2	Breast cancer gene
CA IX	Carbonic anhydrase IX
CagA	Cytotoxin-associated gene A
CEA	Carcinoembryonic antigen
CISH	Chromogenic in situ hybridization
c-myc2	Cellular myelocytomatosis 2
COX-2	Cytochrome Oxidase Subunit 2
CT	Computed tomography
D-GC	Diffuse type gastric cancer
DISH	Dual-color in situ hybridization
EEA-EFTA	The European Economic Area / European Free Trade Association
EGFR	Human epidermal growth factor receptor
EMR	Endoscopic mucosal resection
ERK	Extracellular signal-regulated kinase
EUS	Endoscopic ultrasound
FAP	Familial adenomatous polyposis
FDA/CAP	Food & Drug Administration/College of American Pathologists
FDG	F-Fluorodeoxyglucose
FDG-PET	F-Fluorodeoxyglucose (FDG)-Positron Emission Tomography (PET)
FISH	Fluorescence in situ hybridization
GC	Gastric cancer
GEJ /EGJ	Gastro-esophageal junction
GERD	Gastroesophageal reflux disease
H & E	Hematoxylin and eosin stain
H. pylori	Helicobacter pylori microorganism
HDGC	Hereditary diffuse gastric cancer
Her2/neu	Human epidermal growth factor receptor 2
HNPCC	Hereditary nonpolyposis colon cancer syndrome

ICD-O	The International Classification of Diseases for Oncology
I-GC	Intestinal type gastric cancer
IHC	Immunohistochemistry
KLF5	Kruppel Like Factor 5
MAPK	Mitogen-activated protein kinase
MMP-2	MMP2 matrix metalloproteinase 2
Mortality ASR	Mortality Age-Standardized Rate (oncology)
MRI	Magnetic resonance imaging
mTOR	Mammalian target of rapamycin
NBI	Magnifying endoscopy with narrow-band imaging
OS	Overall survival
PET	Positron Emission Tomography
PI3K	Phosphoinositide 3-Kinase
PKP3	Plakophilin-3
ROS	Reactive oxygen species
RT	Radiation therapy
RTKs	Receptor tyrosin-kinases
SATB1	SATB homeobox 1
SISH	Silver in situ hybridization
TGF β	Transforming Growth Factor beta
TNM	The tumor-node-metastasis system
ToGA trial	Trastuzumab for GAstric cancer trial
TOP2A	Topoisomerase II alpha
VEGF	Vascular Endothelial Growth Factor
WHO/IARC	World Health Organization International Agency for Research on Cancer

List of tables

<i>Table</i>		<i>Page</i>
Table (1)	Histologic Artifacts Related to Endoscopy (Endoscopy-Induced Artifacts)	16
Table (2)	Immunohistochemical markers in GC	48
Table (3)	Comparison of differences between Her2/neu scoring in gastric and breast cancer	52
Table (4)	Her2/neu scoring criteria for GC	55
Table (5)	Clinicopathologic characteristics of the studied gastric carcinoma cases	74
Table (6)	Age of patients according to Her2/neu expression by IHC in carcinoma cases	76
Table (7)	The relation between tumor histologic type and Her2/neu expression by IHC in carcinoma cases	77
Table (8)	The relation between tumor grade and Her2/neu expression by IHC in carcinoma cases	78
Table (9)	Tumor depth of invasion in relation to Her2/neu expression by IHC in carcinoma cases	79
Table (10)	Lymph node status in relation to Her2/neu expression by IHC in carcinoma cases	80
Table (11)	Metastatic status in relation to Her2/neu expression by IHC in carcinoma cases	81
Table (12)	Location of the tumor in relation to Her2/neu expression by IHC in carcinoma cases	82
Table (13)	Immunohistochemistry- Fluorescence in-situ hybridization concordance in gastric carcinoma cases	83
Table (14)	Age of the patients in different lesions (Mean \pm standard deviation, range)	86
Table (15)	Her2/neu expression in cases of gastritis, intestinal metaplasia, both low and high grade dysplasia and adenocarcinoma	87
Table (16)	The relation between histologic type and Her2/neu gene amplification by FISH	88
Table (17)	The relation between tumor grade and Her2/neu gene amplification by FISH	89

Table (18)	The relation between tumor depth of invasion and Her2/neu gene amplification by FISH	90
Table (19)	The relation between lymph node status and Her2/neu gene amplification by FISH	91
Table (20)	The relation between metastatic status and Her2/neu gene amplification by FISH	92
Table (21)	The relation between tumor location and Her2/neu gene amplification by FISH	93

List of graphs

<i>Graph</i>		<i>Page</i>
Graph (1)	Her2/neu protein expression in all carcinoma cases	75
Graph (2)	Age of patients according to Her2/neu expression by IHC in carcinoma cases.	76
Graph (3)	The relation between tumor histologic type and Her2/neu expression by IHC in carcinoma cases	77
Graph (4)	The relation between tumor grade and Her2/neu expression by IHC in carcinoma cases	78
Graph (5)	Tumor invasion in relation to Her2/neu expression by IHC in carcinoma cases	79
Graph (6)	Lymph node status in relation to Her2/neu expression by IHC in carcinoma cases	80
Graph (7)	Metastatic status in relation to Her2/neu expression by IHC in carcinoma cases	81
Graph (8)	Location of the tumor in relation to Her2/neu expression by IHC in carcinoma cases	82
Graph (9)	IHC –FISH concordance in gastric carcinoma cases	83
Graph (10)	Her2/neu expression in gastric carcinoma cases (IHC & FISH results).	84
Graph (11)	Mean age of the patients in different lesions	86
Graph (12)	Her2/neu expression in cases of gastritis, intestinal metaplasia, both low and high grade dysplasia and adenocarcinoma	87
Graph (13)	The relation between histologic type and Her2/neu gene amplification by FISH	88
Graph (14)	The relation between tumor grade and Her2/neu gene amplification by FISH	89
Graph (15)	The relation between tumor depth of invasion and Her2/neu gene amplification by FISH	90
Graph (16)	The relation between lymph node status and Her2/neu gene amplification by FISH	91
Graph (17)	The relation between metastatic status and Her2/neu gene amplification by FISH	92
Graph (18)	The relation between tumor location and Her2/neu gene amplification by FISH	93

List of Figures

<i>Figure</i>		<i>Page</i>
Figure (1)	Country-specific incidence rates of gastric cancer	5
Figure (2)	Correa model for intestinal type GC	21
Figure (3)	Carneiro's model for diffuse type GC	21
Figure (4)	Anatomic location for subsets of GC	23
Figure (5)	Her2/neu signaling pathway and interaction with other pathways and examples of classes of drugs targeting the Her2/neu network	34
Figure (6)	The World Health Organization GLOBOCAN database, Mortality ASR	35
Figure (7)	Her2/neu protein: schematic representation.	39
Figure (8)	Receptor dimerization	40
Figure (9)	Signal transduction by the HER family	41
Figure (10)	Her2/neu gene amplification in cancer cell compared to normal cell	43
Figure (11)	Stepwise approach to IHC scoring in GC: tissue and quality issues	57
Figure (12)	Her2/neu gene	68
Figure (13)	The FISH and molecular unit, Theodor Bilharz Research Institute	72
Figure (14)	A case of well differentiated gastric adenocarcinoma; malignant glands infiltrate perigastric fat (arrow) (H&E x100).	94
Figure (15)	A case of well differentiated gastric adenocarcinoma; malignant cells show mild nuclear anaplasia and arranged in well-formed glands (H&E x400).	94
Figure (16)	A case of moderately differentiated gastric carcinoma shows papillary architecture with fibrovascular cores and distorted glands (H&E x100).	95
Figure (17)	A higher power from the previous case shows moderately differentiated gastric adenocarcinoma, shows papillary architecture with fibrovascular cores covered by epithelial cells show moderate nuclear anaplasia (H&E x200).	95
Figure (18)	A case of moderately differentiated gastric adenocarcinoma, arranged in glandular and cribriform patterns with intense lymphoid stroma forming lymphoid follicles (H&E x40).	96
Figure (19)	A higher power from the previous case of moderately differentiated gastric adenocarcinoma with intense lymphoid stroma (H&E x100).	96
Figure (20)	A case of poorly differentiated gastric adenocarcinoma, malignant cells mostly arranged in solid sheets (H&E x100).	97

Figure (21)	A higher power from the previous case of poorly differentiated gastric adenocarcinoma, malignant cells show moderate nuclear anaplasia (H&E x400).	97
Figure (22)	A case of poorly differentiated gastric adenocarcinoma, arranged mainly in solid sheets (H&E x50).	98
Figure (23)	A higher power from the previous case of poorly differentiated adenocarcinoma, formed of solid sheet of tumor cells (H&E x400).	98
Figure (24)	A case of gastric signet ring cell carcinoma; shows signet ring cells with eccentric hyperchromatic nuclei and intracellular mucin, diffusely infiltrate in between gastric acini with focal intestinal metaplasia (H&E x200).	99
Figure (25)	A case of moderately differentiated adenocarcinoma at gastro-esophageal junction, malignant glands interrupt the esophageal mucosa (H&E x100)	99
Figure (26)	Section from gastric mucosa shows evidence of gastritis (H&E x100).	100
Figure (27)	Non-tumorous mucosa adjacent to malignancy in a case of gastric adenocarcinoma shows intestinal metaplasia (H&E x200).	100
Figure (28)	A case of adenoma with low grade dysplasia, the gastric acini show hyperchromatic, elongated, atypical nuclei and mucin depletion (H&E x400).	101
Figure (29)	A case of adenoma with high grade dysplasia, showed irregular glands with marked nuclear stratification (H & E x 200).	101
Figure (30)	A case of well differentiated gastric adenocarcinoma shows negative malignant glands for Her2/neu expression (Score 0) (IHC, DAB, x100).	102
Figure (31)	A case of moderately differentiated gastric adenocarcinoma, showing malignant glands are negative for Her2/neu expression (Score 0) (IHC, DAB, x200)	102
Figure (32)	A case of moderately differentiated gastric adenocarcinoma, malignant glands show barely visible Her2/neu protein expression (Score 1+) (IHC, DAB, x200).	103
Figure (33)	A higher power of the previous case of moderately differentiated gastric adenocarcinoma, malignant glands show visible weak Her2/neu protein expression at high power (Score 1+) (IHC, DAB, x400).	103
Figure (34)	A case of poorly differentiated gastric adenocarcinoma, malignant cells show moderate membranous Her2/neu protein expression (Score 2+) (IHC, DAB, x100).	104

Figure (35)	A case of moderately differentiated gastric adenocarcinoma, malignant glands show moderate membranous basolateral and complete Her2/neu protein expression (Score 2+) (IHC, DAB, x200).	104
Figure (36)	A case of moderately differentiated gastric adenocarcinoma, malignant cells show moderate membranous basolateral and lateral staining for Her2/neu (Score 2+) (IHC, DAB, x400).	105
Figure (37)	A case of moderately differentiated adenocarcinoma with papillary pattern shows strong membranous staining for Her2/neu at low power (Score 3+) (IHC, DAB, x50).	105
Figure (38)	Higher power from the previous case of moderately differentiated gastric adenocarcinoma with papillary pattern shows strong membranous staining for Her2/neu (Score 3+) (IHC, DAB, x200).	106
Figure (39)	Higher power from the previous case of adenocarcinoma with papillary pattern shows strong complete, basolateral or lateral membranous staining for Her2/neu (Score 3+) (arrow) (IHC, DAB, x400).	106
Figure (40)	A case of signet ring cell carcinoma showing diffusely infiltrating malignant cells positive for Her2/neu (Score 3+) (IHC, DAB, x100).	107
Figure (41)	Higher power of the previous case of signet ring cell carcinoma, malignant cells are positive for Her2/neu (Score 3+) (IHC, DAB, x400).	107
Figure (42)	A case of moderately differentiated gastric adenocarcinoma showing heterogeneous expression of Her2/neu, some glands show moderate membranous staining (Score 3+) (red arrow), others are negative for Her2/neu (Score 0) (green arrow) (IHC, DAB, x100).	108
Figure (43)	A case of moderately differentiated gastric adenocarcinoma showing heterogeneous expression of Her2/neu, some malignant cells show moderate membranous staining (Score 2+) (black arrow), others are negative for Her2/neu (Score 0) (red arrow) (IHC, DAB, x100).	108
Figure (44)	Non-tumorous gastric mucosal glands adjacent to the malignancy show moderate staining for Her2/neu in a case of gastric adenocarcinoma (IHC, DAB, x50).	109
Figure (45)	Non-tumorous gastric mucosal glands adjacent to the malignancy show weak staining for Her2/neu in a case of gastric adenocarcinoma (IHC, DAB, x200).	109
Figure (46)	Section from gastric mucosa with gastritis, is negative for membranous immunostaining for Her2/neu (IHC, DAB, x200)	110

Figure (47)	Gastric acini with intestinal metaplasia show weak Her2/neu expression (IHC, DAB, x200).	110
Figure (48)	Dysplastic acini negative for Her2/neu expression (Score 0), in a case of gastric adenocarcinoma (IHC, DAB, x100).	111
Figure (49)	Dysplastic acini with weak Her2/neu expression (Score 2+) in a case of gastric adenocarcinoma (IHC, DAB,x200).	111
Figure (50)	Dysplastic acini with moderate Her2/neu expression (Score 2+) in a case of gastric adenocarcinoma (IHC,DAB, x200).	112
Figure (51)	Dysplastic mucosa shows strong Her2/neu expression (score 3+) in a case of gastric adenocarcinoma (IHC, DAB, x200).	112
Figure (52)	A case of moderately differentiated gastric adenocarcinoma, negative for Her2/neu gene amplification, IHC score 2+ (FISH, Her2/neu gene,x1000).	113
Figure (53)	A case of moderately differentiated gastric adenocarcinoma, positive for Her2/neu gene amplification, showing red clusters (orange arrow), IHC score 2+ (FISH, Her2/neu gene, x1000).	113
Figure (54)	A case of moderately differentiated gastric adenocarcinoma, positive for Her2/neu gene amplification, showing red clusters (orange arrow), IHC score 3+ (FISH, Her2/neu gene, x1000).	114

LIST OF CONTENTS

<i>Title</i>	<i>Page</i>
Introduction	1
Aim of the work	3
Review of literature.....	4
Gastric cancer	4
Incidence	4
Epidemiology and predisposing causes	7
Sex distribution	7
Age distribution	7
Risk factors	7
<i>Diet</i>	8
<i>Obesity</i>	9
<i>Smoking</i>	9
<i>Helicobacter pylori (H. pylori) infection</i>	9
<i>Radiation exposure</i>	12
<i>Previous gastric surgery</i>	12
<i>Genetic Factors</i>	13
Diagnosis	14
Clinical Manifestation	14
Screening for Gastric Cancer	15
Diagnosis and Clinical Staging	16
Pathology of gastric cancer	19
<i>WHO histological classification of gastric tumors</i>	19
<i>Pathogenesis</i>	20
<i>Gross Appearances</i>	23
<i>Microscopic Appearances</i>	24
<i>Grading of gastric carcinoma</i>	26
<i>Pathologic TNM Staging</i>	26
<i>Definitions of TNM of stomach cancer</i>	26
<i>Definitions of TNM of Esophagus and Esophagogastric Junction</i>	27
Treatment of Gastric Cancer	29
Endoscopic Resection	29
Surgery & lymphadenectomy	29
Chemotherapy	30
Adjuvant Therapy	30

Radiation Therapy	31
Molecular Targeted Agents	31
Prognosis of Gastric Cancer	35
Prognostic features	36
Human epidermal growth factor receptor 2	38
Physiological basal function in normal cell	41
Her2/neu Gene And Tumorigenesis	43
Identification of Her2/neu status	44
Her2/neu And Gastric Carcinoma	46
Standardized methodology and scoring criteria of	
Her2/neu assessment in gastric cancer	49
The ToGA Trial (trastuzumab for GAstric cancer study....	50
<i>Her2/neu Testing in the ToGA Trial</i>	50
Heterogeneity	53
Practical guidance on Her2/neu testing in gastric cancer	53
Sample Analysis: Immunohistochemistry	54
Stepwise approach to IHC scoring in gastric cancer	57
Incidence of Her2/neu expression in gastric cancer.....	58
Relation between Her2/neu overexpression and	
gastric cancer prognosis.....	60
 Material & methods	 62
Results.....	73
Discussion.....	115
Summary.....	130
Conclusions & recommendations	132
References.....	134
Arabic summary	